BIOCHEMICAL AND KARYOTYPIC EVIDENCE FOR THE SPECIFIC STATUS OF THE RODENT *LEGGADINA LAKEDOWNENSIS* WATTS

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Summary

BAVERSTOCK, P. R., HOGARTH, J. T., COLE, S., & COVACEVICH, J. (1976).—Biochemical and karyotypic evidence for the specific status of the rodent *Leggadina lakedownensis* Watts. *Trans. R. Soc. S. Aust.* **100**(2), 109-112, 31 May, 1976.

Leggadina lakedownensis Watts differs karyotypically from its apparent nearest relative, *L. forresti*. Further, the biochemical differences between *L. lakedownensis* and *L. forresti* are greater than those between other "good" species of similar sized pseudomyins. These data support the specific status of *L. lakedownensis*.

Introduction

In 1973 several specimens of a species of small rodent were collected from Lakeland Downs in northeast Queensland and described as a new species, *L. lakedownensis*, by Watts (1976). The morphological differences between *L. lakedownensis* and its apparent nearest relative *L. forresti* are, however, minor. Because speciation is the result of the accumulation of many genetic differences and because morphology alone reflects only part of these genetic differences, it seems desirable in such cases to assess other aspects of genetic differences between allopatric populations. The present study was undertaken to determine whether the karyotypic and biochemical differences between *L. forresti* and *L. lakedownensis* substantiated the recognition of the latter as a distinct species.

Methods

Sources of animals: The sources of animals utilized in the present study are shown in Table 1, along with their Institute of Medical and Veterinary Science number. When these animals die their skull and skin will be submitted to a Museum and given a Museum number. Museum numbers corresponding to IMVS numbers will be available from the IMVS or the South Australian Museum.

Chromosome preparations: Chromosome preparations were made from 8 *L. forresti* and 3 *L. lakedownensis*. Animals were bled by cardiac puncture under ether anaesthesia and leukocytes cultured for 3 or 4 days. Slides were prepared by means of the routine

TABLE 1

Reference numbers and sources of animals

Species	IMVS No.	Sex	Locality
(a) Chromosomes			
L. forresti	1	F	Coorabulka, SW Qld
	2	M	19 km W Innamincka, S.A.
	3	F	19 km W Innamincka, S.A.
	5	F	19 km W Innamincka, S.A.
	6	M	Fowlers Gap Stn, N.S.W.
	7	M	Fowlers Gap Stn, N.S.W.
	8	M	Fowlers Gap Stn, N.S.W.
	9	M	Mt Sarah Stn, S.A.
L. lakedownensis	10*	F	Lakeland Downs Stn, Qld
	11	M	Lakeland Downs Stn, Qld
	12	M	Lakeland Downs Stn, Qld
(b) Electrophoresis			
L. forresti	3	F	19 km W Innamincka, S.A.
	5	F	19 km W Innamincka, S.A.
L. lakedownensis	10*	F	Lakeland Downs Stn, Qld
L. delicatula	13	M	Fairbairn Dam, 22 km SW Emerald, Qld
	14	M	Fairbairn Dam, 22 km SW Emerald, Qld
L. hermannsburgensis	14	M	Lab. stock
P. novaehollandiae	15	M	Port Stevens area, N.S.W.
P. australis		M	Lab. stock

* Holotype—Queensland Museum JM1292.

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TABLE 2

Electrophoretic buffer and staining systems used for proteins examined

Enzyme/Protein	Buffer system	Stain
E.E. 1.1.1.49 Glucose 6-Phosphate dehydrogenase (G 6-PD)	Brewer (1970)	Brewer (1970)
6-Phosphogluconate dehydrogenase E.C. 1.1.1.44 (6-PGD)	Brewer (1970)	Brewer (1970)
Phospho-hexose isomerase E.C. 5.3.1.9. (PHI)	Selander *et al.* (1971) 7	Brewer (1970)
Phosphoglucomutase E.C. 2.7.5.1. (PGM)	Selander *et al.* (1971) 7	Brewer (1970)
Lactate dehydrogenase E.C. 1.1.1.27 (LDH)	Holmes *et al.* (1973) TEB*	Brewer (1970)
NAD-Malate dehydrogenase E.C. 1.1.1.37 (NAD-MDH)	Holmes *et al.* (1973) TEB*	Brewer (1970)
Leucine aminopeptidase E.C. 3.4.1.1 (LAP)	Selander *et al.* (1971) 2	Brewer (1970)
Glyceraldehyde 3-Phosphate dehydrogenase GA 3-PD E.C. 1.2.1.12	Brewer (1970)	Brewer (1970)
Tetrazolium oxidase	Brewer (1970)	Scored from gels stained from GA 3-PD
Esterase (Est)	Sol^n A: 0.08M. tris-citrate pH 8.6 Sol^n B: 0.06 M. Li-borate-pH 8.8 Gel: 337.5 ml Sol^n A: 62.5 ml Sol^n B in 400 ml B. Electrode: Sol^n B.	Brewer (1970)
Albumin (Alb.)	as for Est	Amido Black
Haemoglobin (Hb)	as for G 6-PD	Amido Black
Transferrin (Tf)	as for Est	Amido Black

* TEB — Tris-EDTA-Borate

air-dry method. For karyotypes, slides were stained with 2% Giemsa. C-staining was conducted by the method of Arrighi and Hsu (1971) except that the RNase step was omitted. Slides were then stained with 10% Giemsa.

Electrophoresis: In addition to *L. forresti* and *L. lakedownensis*, specimens of *L. delicatula*, *L. hermansburgensis*, *Pseudomys novaehollandiae* and *P. australis* were studied. Blood was collected by cardiac puncture under ether anaesthesia in syringes containing a dried film of heparin and centrifuged immediately at 2,000G for 10 minutes at room temperature. Plasma was pipetted off and stored at −20°C. The red cells were washed 3 times in 2 volumes of isotonic saline and lysed in an equal volume of distilled water containing 1/5 volume of toluene. Cell walls were centrifuged out and the clear supernatant stored at −20°C for a maximum period of 3 weeks.

Horizontal starch gell electrophoresis was used. Gel slabs 300 mm x 150 mm x 6 mm were prepared from 12.5% (50g/400 ml buffer) starch using a perspex mould. Gels were run in a refrigerator to minimize heating. After electrophoresis a section approximately 90 mm x 120 mm was cut from the gel slab, sliced into two separate halves, and these halves then incubated in the appropriate staining solutions (Table 2).

Results

Chromosome studies: Fig. 1a shoks the karyotype of a female *L. forresti.* The diploid number was 2N = 48. The largest chromosome, designated pair 1, was acrocentric in IMVS 1, 2, 5, 7, 8 and 9. However, in IMVS 3 and 6, pair 1 was heteromorphic, one member being acrocentric and the other being subacrocentric with a distinct short arm. Pairs of 2 to 21 were acrocentric forming a series graded in size. Pairs 22 and 23 were small metacentrics. The presumed X-chromosome was an acrocentric representing about 6% of the total chromosome length and the Y-chromosome an acrocentric.

The C-staining technique (Fig. 1b) showed that although the centrometric area of some chromosomes were C-banded, only in the small metacentric pairs 22 and 23 was this marked. In many preparations the presumed Y stained slightly more intensely than other chromosomes over its entire length.

Chromosomally *L. lakedownensis* differs from *L. forresti* in possessing only one pair of small metacentrics (Fig. 1c). Also, in the three individuals karyotyped, pair 1 was always acrocentric. Centromeric C-banding of *L. lake-*

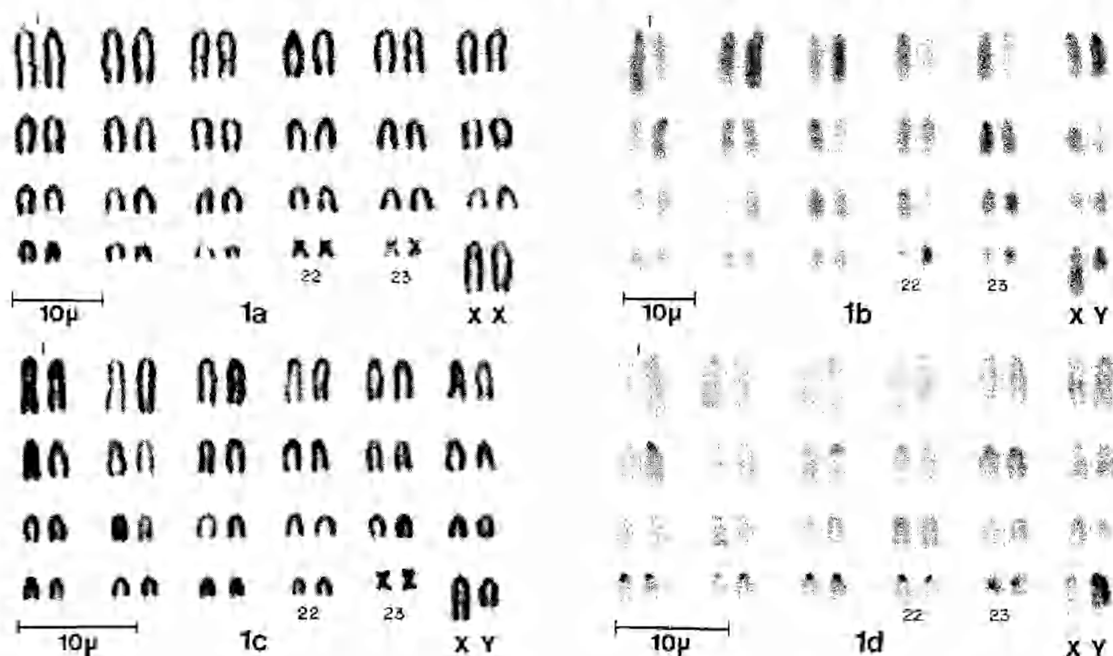

Fig. 1. Karyotype and C-banding of *L. forresti* and *L. lakedownensis*. 1a, Karyotype of female *L. forresti* (IMVS 3) heteromorphic for pair 1; 1b, C-banding of male *L. forresti* (IMVS 6) heteromorphic for pair 1; 1c, Karyotype of male *L. lakedownensis* (IMVS 11); 1d, C-banding of male *L. lakedownensis* (IMVS 12).

Species	G-6-PD	Hb	TO	GA-3-PD	Est-1	Alb	NAD-MDH
L. f	I	I	I	I	I	I	I
L. l	I	I	I	I	I	I	I
L. d	I	II	I	I	I	I	I
L. h	I	II	I	I	I	I	I
P. n	I	II	I	I	I	I	I
P. a	I	I	III	I	I	I	I

Fig. 2. Representation of electrophoretic patterns observed for seven useful proteins in six pseudomyin species. In each case the origin is to the left, and fastest migrating bands to the right (cathodal). Key—*L.f.* = *Leggadina forresti; L.l.* = *L. lakedownensis; L.d.* = *L. delicatula; L.h.* = *L. hermannsburgensis; P.n.* = *Pseudomys novaehollandiae; P.a.* = *P. australis.*

downensis (Fig. 1d) was evident only in the smaller chromosomes. The presumed Y was slightly more intensely C-banded than other chromosomes over its entire length.

Electrophoresis: Of the 16 proteins studied (Table 2), LAP, PHI, Tf, Est. 2 and Est. 3 showed evidence of polymorphism in at least one species, and 6-PGD, LDH A & B and PGM were identical for all species. This left seven of the proteins studied that were consistent within species but varied between species. The electrophoretic results for these seven proteins are shown in Fig. 2, and the resulting difference matrix in Table 3.

TABLE 3
Difference matrix for data in Figure 2
(Key as in Figure 2)

	L.f.	*L.l.*	*L.d.*	*L.h.*	*P.n.*	*P.a.*
L.f.	0					
L.l.	3	0				
L.d.	6	5	0			
L.h.	6	5	0	0		
P.n.	7	6	1	1	0	
P.a.	6	7	4	4	4	0

Discussion

Most *L. forresti* were found to possess the same karyotype, although two were heteromorphic for a sub-acrocentric pair I. C-banding showed that the short arm on the sub-acrocentric member was not heterochromatic, suggesting that the sub-acrocentric was related to the acrocentric by a pericentric inversion.

L. lakedownensis, however, had a pair of small metacentrics converted to a pair of acrocentrics, presumably by a pericentric inversion. Although a single fixed chromosomal difference between *L. forresti* and *L. lakedownensis* is insufficient in itself to indicate a species difference, taken in the context of the very low karyotypic variation of the whole of the pseudomyinae (unpublished data), a single chromosomal rearrangement probably indicates reasonable differentiation.

The biochemical data are more convincing. Of the seven useful proteins studied, *L. forresti* and *L. lakedownensis* differ in 3 (Table 3). This is considerable compared to the biochemical differentiation between 3 "good" species—*L. delicatula* and *L. novaehollandiae* (1 difference), and *L. novaehollandiae* and *L. hermannsburgensis* (1 difference). These results suggest that *L. forresti* and *L. lakedownensis* may have been separated from each other for at least as long as have *L. delicatula*, *L. novaehollandiae* and *L. hermansburgensis*.

Phenetically *L. lakedownensis* and *L. forresti* are biochemically more similar to each other than either is to any other pseudomyin studied (Table 3). Although more data are needed these results support the maintenance of *Leggadina* as a separate genus which at this time would include only these two species.

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